(ii) ''Purified Protein Derivative'' or ''PPD.''

(c) *Samples; protocols; official release.* For each lot of Tuberculin the following shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(2) Tuberculin distributed on a multiple puncture device, as follows:

(i) A total of no less than 50 devices.

(ii) A total of no less than 6 milliliters of bulk tuberculin.

(3) A total of no less than 20 ml. of liquid tuberculin.

(4) Sufficient dried tuberculin in final containers so that upon reconstitution as recommended in labeling it will yield at least 20 milliliters.

(5) The product shall not be issued by the manufacturer until written notification of official release of the lot is received from the Director, Center for Biologics Evaluation and Research.

[38 FR 32097, Nov. 20, 1973, as amended at 39 FR 9661, Mar. 13, 1974; 42 FR 27584, May 31, 1977; 42 FR 54546, Oct. 7, 1977; 49 FR 23834, June 8, 1984; 51 FR 15611, Apr. 25, 1986; 55 FR 11013, Mar. 26, 1990]

§ 650.12 U.S. Standard preparations.

(a) The U.S. Standard Tuberculin, Old, shall be used for determining the potency of nonfractionated tuberculins, as prescribed in § 650.14. One U.S. Tuberculin unit is 0.1 ml. of a 1:10,000 dilution of this standard.

(b) The U.S. Standard Tuberculin, Purified Protein Derivative, shall be used in determining the potency of tuberculins made from protein fractions, as prescribed in § 650.14. One U.S. Tuberculin unit is 0.1 ml. of a 1:5,000 dilution of this standard.

§ 650.13 Production.

(a) *Propagation of mycobacteria.* The medium used for production of mycobacteria shall not contain ingredients known to be capable of producing allergenic effects in human subjects.

(b) *Tests for viable mycobacteria.* The culture filtrate from each strain in its most concentrated form shall be shown to be free of viable mycobacteria by the following tests:

(1) *Animal test.* A 1.0 ml. sample of the filtrate shall be injected intraperitoneally into each of at least three healthy guinea pigs weighing between 300 and 400 gm. At least two-thirds of the animals must survive an observation period of at least 6 weeks and must show a normal weight gain. After the observation period the animals shall be necropsied and examined for signs indicative of tuberculosis except that animals that die during the observation period shall be necropsied and examined as soon as feasible after death. The filtrate is satisfactory for Tuberculin manufacture if none of the animals in the test show evidence of tuberculosis infection.

(2) *Culture test.* A 2.0 ml. sample of the filtrate shall be inoculated onto Lowenstein-Jensen's egg medium or other media demonstrated to be equally capable of supporting growth. A control test on the culture medium shall be conducted simultaneously with the sample under test and shall be shown to be capable of supporting the growth of small numbers of the production strain(s). All the test vessels shall be incubated at a suitable temperature for a period of 6 weeks under conditions that will prevent drying of the medium, after which the cultures shall be examined for evidence of mycobacterial colonies. The filtrate is satisfactory for Tuberculin manufacture if the test shows no evidence of mycobacteria.

(c) *Chemical characterization.* Each batch of powdered tuberculin material shall be chemically characterized, including protein, carbohydrate, lipid, and nucleic acid content to assess consistency of production.

[38 FR 32097, Nov. 20, 1973, as amended at 44 FR 40289, July 10, 1979]

§ 650.14 Potency testing in animals.

The potency of each lot of Tuberculin shall be estimated from a comparison of the responses obtained by the intradermal injection into sensitized guinea pigs weighing over 500 gm. of a sample of the lot under test and of the